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In-vitro evaluations of *Trichoderma* spp. against Different Diseases of Pigeonpea in Nagaland

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Abstract

The investigation was carried out at the laboratory of Plant Pathology Department, SASRD, Nagaland University (NU), Medziphema Campus, Nagaland, India during the year kharif (August-October, 2020) to evaluate the antagonistic potentiality of *Trichoderma* spp. (*T. harzianum*, *T. asperellum* and *T. virens)* against three diseases of pigeonpea viz. Alternaria leaf spot (*Alternaria alternata*), Collar rot (*Sclerotium rolfsii*), Phytophthora blight (*Phytophthora drechsleri* f. sp. *cajani*). Dual culture technique was followed to test the efficacy of these antagonists as bio-agents against the pathogens. Result of *in vitro* screening test revealed that all the bio-agents significantly inhibited the mycelial growth of the pathogens. Among the three *Trichoderma* spp. evaluated, *T. harzianum* (55.53%) was found to be the highest in per cent inhibition of *A. alternata*, *T. asperellum* (50.87 %) recorded highest per cent inhibition on *S. rolfsii* and *T. virens* (55.88%) recorded highest per cent inhibition on *Phytophthora drechsleri* f. sp. *cajani*. Thus, the *in vitro* study suggests that the *Trichoderma* spp. has a potential to be used as bio-agents in managing these three fungal diseases viz. Alternaria leaf spot, Collar rot and Phytophthora blight of pigeonpea.

Keywords: Bio-agents, pigeonpea, *Trichoderma*, efficacy, inhibition, pathogen

1. Introduction

In India, pulses are one of the important group of crops which is responsible for yielding large financial gains by amounting for a large part of the exports. India ranks first in area and production with 31% and 28% respectively and is the largest pulse producing country in the world. Under pulses crop, pigeonpea is the second highest contributor with 17% share to total pulses production (Anonymous, 2022). It is also the sixth most important legume crop in the world (Anonymous, 2014). It is not only an important source of protein, but also plays a vital role in atmospheric nitrogen fixation into soil (Reddy et al., 2012). India has largest acreage under Pigeonpea (4.71 mha) with a total production and productivity of 4.13 mt and 877 kg ha^{-1}, respectively (Anonymous, 2022). According to statistical hand book of Nagaland, pigeonpea constitutes an area of 3210 ha in the state with the production of 2920 mt (Anonymous, 2020). Several biotic and abiotic factors are responsible for reducing the yield (Anonymous, 2018). Diseases are a major biological causes and the crop suffers from dreaded fungal, viral and bacterial diseases resulting in tremendous losses in yield. Alternaria leaf spot of pigeonpea is caused by *Alternaria* spp. (A. *alternata* and *A. tenussima*). The plants infected by this pathogen tends to show symptoms on aerial parts of the plant such as stem, leaves and pods. Leaf spot is characterized as black-brownish with prominent black dots in severe infection and diseased plants are blighted due to the coalescing of the several spots in tender shoots and leaves. Collar rot caused by *Sclerotium rolfsii*, is a very important polyphagus pathogenic fungus causing considerable losses in quality and productivity of yield (Arya et al., 2021). Under suitable climatic condition collar region of the infected plant shows white mycelial growth of the fungus and sometimes initials of sclerotia were also observed under *in vivo* condition (Awasthi et al., 2018). Phytophthora blight disease is a major disease of pigeonpea which is caused by *Phytophthora drechsleri* f. sp. *cajani*. It is soil borne and the pathogen overwinter as chlamydospores, oospores and dormant mycelium in soil as well as on plant debris (Reddy et al., 2012). Examining the constraints in pulse production the target should be in minimizing huge losses to these diseases. *Trichoderma* spp. are being used broadly and gained importance as biocontrol agent against plant pathogens due to its efficacious nature against a variety of pathogen and for its growth. Literature reports the role of *Trichoderma* as bio-control agents against diseases of leaves spots that could be of importance for disease management (Prasad et al., 2013). Inhibitory activity of *Trichoderma* spp. against *A. alternata* has been reported my many workers (Rani, 2017, Marchande et al., 2020, Gatak et al., 2020, Ambuse and Bhale, 2015, Khillare et al., 2022). Efficacy of *Trichoderma* spp. against the growth of *S. rolfsii* have also been reported for management of Collar

rot disease (Prajapati et al., 2015, Salvi et al., 2017, Sumi, 2013, Sumi et al., 2018, Kushwaha et al., 2018). *Trichoderma* spp. are being used as biocontrol agents against *Phytophthora* species (Wang et al., 2013). Studies suggest that *Trichoderma* spp. has a potential to be used as bio-control agents against *Phytophthora drechsleri* f. sp. *cajani* (Jadesha et al., 2019). Among the antagonistic fungi, *Trichoderma* species are the most studied bio-agents in pulse ecosystem (Mishra et al., 2017). With this in background, the present investigation was carried out to study the efficacy of *Trichoderma* spp. against these three diseases of pigeonpea crop.

2. Materials and Methods

Present investigation was carried out at the laboratory of Plant Pathology Department, SASRD, Nagaland University (NU), Medziphema Campus, Nagaland, India during the year kharif (August-October, 2020).

2.1. Pathogens and antagonist

Three fungal antagonists of *Trichoderma* spp. viz. *T. harzianum, T. asperellum and T. virens* were procured from Plant Pathology Department, SASRD, NU, Medziphema Campus and the three pathogens were isolated from natural source of infection during the year *kharif* 2020–2021 collected from Research Farm of AICRP, SASRD, Nagaland University (NU), Medziphema Campus.

2.2. In vitro evaluation of bio-agents against Alternaria alternata, Sclerotium rolfsii and Phytophthora drechsleri f. sp. *cajani*.

To evaluate the antagonistic potential of bio-agents against the pathogen, *in vitro* experiment was carried out by dual culture method at the laboratory of Plant Pathology Department, SASRD, Nagaland University (NU), Medziphema campus during the year kharif 2020–2021. The experiment was conducted at CRD and each treatment was replicated thrice. In solidified PDA media, mycelial disc of pathogen and bio-agents each of (5 mm dia) were cut out with sterilized cork borer and then these two culture discs were placed opposite to each other aseptically and plates were incubated at 26±2°C. A control plate having test pathogen was maintained for comparison. Linear mycelial growth observations on the test pathogen and test bio-agent were recorded at an interval of 24 hours. Growth inhibition rate given by Vincent (1947) was calculated by formula:

$$GI (\%) = (C-T)/C \times 100 \quad \text{..... (1)}$$

Where, GI = Growth inhibition (%) of the pathogen
C=Growth of the pathogen in control plate
T=Growth of the pathogen in dual culture plate

3. Results and Discussion

3.1. Antagonistic effect of Trichoderma spp. *on mycelial growth of Alternaria alternata*, *Sclerotium rolfsii and Phytophthora drechsleri* f.sp *cajani*

Antagonistic effect of *Trichoderma* spp. on *Alternaria alternata*, *Sclerotium rolfsii* and *P. drechsleri* f.sp *cajani* was tested on PDA in dual culture method. The *Trichoderma* spp. showed significant effect on the radial growth and per cent inhibition in all the three pathogens. Differential actions of the bio-agents were noticed on the mycelial growth of the pathogen at different interval of incubation. Among the *Trichoderma* spp. *T. harzianum* was found to be highest in per cent inhibition (55.53%) followed by *T. virens* (51.23%) and *T. asperellum* (42.12%) respectively on mycelial growth of *A. alternata* (Table 1, 2 and Plate 1).

Table 1: *In vitro* evaluation of bio-agents against mycelial growth of *A. alternata*

Treatments	24 hrs	48 hrs	72 hrs	Mean
T_1 (*A. alternata*+*T. harzianum*)	3.33	6.33	11.33	7.00
T_2 (*A. alternata*+*T. asperellum*)	4.67	8.33	13.33	8.78
T_3 (*A. alternata*+*T. virens*)	3.67	7.00	12.33	7.67
T_0 Control (*A. alternata*)	7.00	14.67	26.50	16.06
SEm±		1.61		
CD (*p*=0.05)		5.58		

Table 2: Per cent inhibition of *A. alternata* in presence of bio-agents

Treatments	24 hrs	48 hrs	72 hrs	Mean
T_1 (*A. alternata*+*T. harzianum*)	52.43	56.83	57.33	55.53
T_2 (*A. alternata*+*T. asperellum*)	33.40	43.23	49.73	42.12
T_3 (*A. alternata*+*T. virens*)	47.71	52.30	53.67	51.23
T_0 Control (*A. alternata*)	0.00	0.00	0.00	0.00
SEm±		2.00		
CD (*p*=0.05)		6.93		

The present findings corroborate with the findings of earlier workers. It was reported that significant inhibition of *A. alternata* was achieved with *T. harzianum* (49.21%). Gatak et al. (2020) and Archana et al. (2020) reported control of this pathogen by *T. asperellum* under *in vitro* conditions. Marchande et al. (2020) reported *T. virens* to be most effective with highest mycelial growth inhibition (62.88%) of the test pathogen *A. alternata*.

Effect on the mycelial growth and per cent inhibition against *S. rolfsii* recorded highest per cent inhibition by *T. asperellum* (50.87%), followed by *T. virens* (47.55%) and *T. harzianum* (40.80%) respectively (Table 3, 4 and Plate 2). The

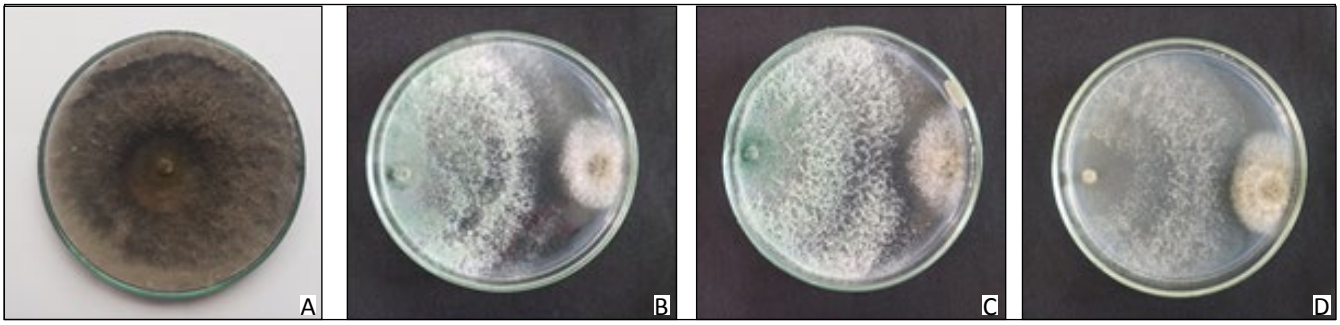

Plate 1: *In vitro* evaluation of bio-agents against *A. alternaria*; (A) T_0: *A. alternaria*; B: *A. alternaria+T. harzianum*; C: *A. alternaria+T. asperellum*; D: *A. alternaria+T. virens*

Table 3: *In vitro* evaluation of bio-agents against mycelial growth of *S. rolfsii*

Treatments	24 hrs	48 hrs	72 hrs	Mean
T_1 (*S. rolfsii+T. harzianum*)	9.33	22.00	31.33	20.89
T_2 (*S. rolfsii+T. asperellum*)	7.67	18.00	26.67	17.45
T_3 (*S. rolfsii+T. virens*)	8.00	19.33	29.00	18.78
T_0 Control (*S. rolfsii*)	15.00	37.00	56.00	36.00
SEm±		2.96		
CD (*p*=0.05)		10.24		

Table 4: Per cent inhibition of *S. rolfsii* in presence of bio-agents

Treatments	24 hrs	48 hrs	72 hrs	Mean
T_1 (*S. rolfsii+T. harzianum*)	37.78	40.54	44.09	40.80
T_2 (*S. rolfsii+T. asperellum*)	48.88	51.35	52.39	50.87
T_3 (*S. rolfsii+T. virens*)	46.66	47.77	48.21	47.55
T_0 Control (*S. rolfsii*)	0.00	0.00	0.00	0.00
SEm±		0.80		
CD (*p*=0.05)		2.77		

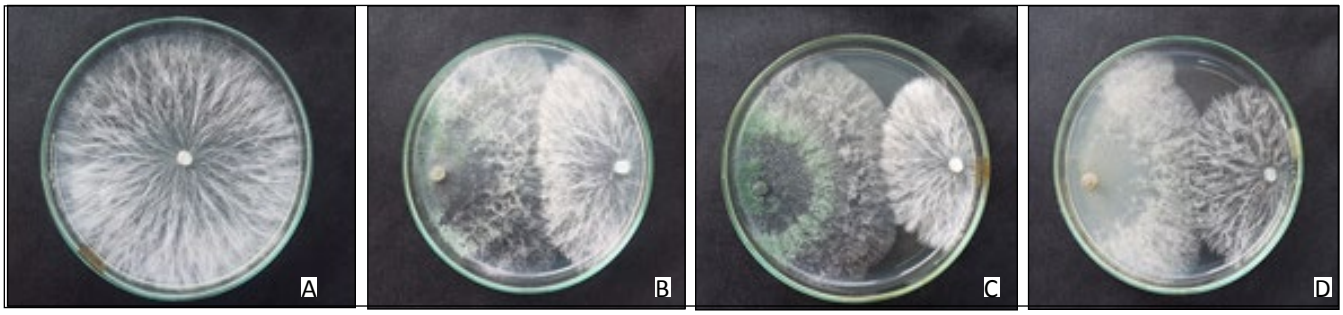

Plate 2: *In vitro* evaluation of bio-agents against *S. rolfsii;* A: T_0 - *S. rolfsii*; B: *S. rolsfii+T. harzianum*; C: *S. rolsfii+T. asperellum* D: *S. rolsfii+T. virens*

findings supported the result of many scientists who found *Trichoderma* spp. effective against *S. rolfsii* (Prajapati et al, 2015 in Chickpea, Sumi and Ao, 2015 in Sunflower, Sumi et al. 2018 in Pigeonpea). Effect on the mycelial growth and per cent inhibition against *P. drechsleri* f. sp. *cajani* recorded highest per cent inhibition by *T. virens* (55.88 %) followed by *T. harzianum* (49.28 %) and *T. asperellum* (44.21%), respectively (Table 5, 6 and Plate 3). Srivastava and Mall (2008) reported significant inhibition by *T. harzianum* (62.5%). Jadesha et al. (2019) and Muhammad et al. (2019) reported efficacy of *T. asperellum* against growth of *P. cajani*.

The growth of the pathogen restricted by bio-agents could have been the resultant action of myco-parasitism, competition and production of antibiotics. *T. harzianum*

Table 5: *In vitro* evaluation of bio-agents against mycelial growth of *Phytophthora drechsleri* f. sp. *cajani*

Treatments	24 hrs	48 hrs	72 hrs	Mean
T_1 (*P. dreschlera* f. sp. *cajani+T. harzianum*)	4.33	11.00	17.33	10.89
T_2 (*P. dreschlera* f. sp. *cajani+T. asperellum*)	5.00	11.67	18.67	11.78
T_3 (*P. dreschlera* f. sp. *cajani+T. virens*)	3.67	9.67	15.33	9.56
T_0 Control (*P. dreschlera* f. sp. *cajani*)	8.00	22.00	36.00	22.00
SEm±		2.21		
CD (*p*=0.05)		7.65		

Table 6: Per cent inhibition of *Phytophthora drechsleri* f. sp. cajani in presence of bio-agents

Treatments	24 hrs	48 hrs	72 hrs	Mean
T_1 (*P. dreschlera* f. sp. *cajani*+*T. harzianum*)	45.83	50.00	52.00	49.28
T_2 (*P. dreschlera* f. sp. *cajani*+*T. asperellum*)	37.50	46.97	48.15	44.21
T_3 (*P. dreschlera* f. sp. *cajani*+*T. virens*)	54.17	56.06	57.40	55.88
T_0 Control (*P. dreschlera* f. sp. *cajani*)	0.00	0.00	0.00	0.00
SEm±		1.45		
CD (*p*=0.05)		5.02		

isolates produces a degrading enzyme chitin and restrict the development of the pathogens (Tozlu et al., 2018). Chernin et al. (1995) and Wright et al. (2001) also reported that these fungal bio-agents deter the development of pathogens by producing antibiotics or enzymes, expeditiously colonizing and competing strongly. Scholars also studied *in vitro* antagonistic effect of four species of *Trichoderma* and reported that toxic fungistatic metabolites may be produced *Trichoderma* species. Inhibitions and parasitism by *Trichoderma* spp. of some species of *P. dreschlera* f. sp*. cajani* have been reported by Elad et al. (1983). Inhibition and mycelial hyphae lysis of the pathogenic fungi have also been reported (Lorito et al., 1994, Paul and Sharma, 2005, Singh and Dubey, 2010).

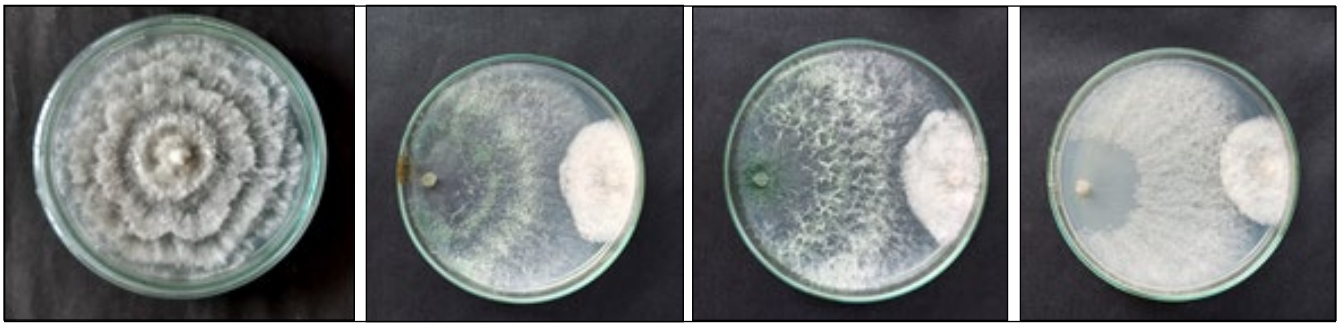

Plate 3: *In vitro* evaluation of bio-agents against *Phytophthora drechsleri* f. sp. *cajani*; A: T_0: *P. drechsleri* f. sp. *cajani* (Pdc); B: Pdc.+*T. harzianum*; C: Pdc+*T. asperellum*; D: Pdc+*T. virens*

4. Conclusion

The *Trichoderma* spp. had a potential to be used as bio-agents against the three pathogens. Studies on *in vivo* application of bio-agents against the pathogen can be further investigated to manage the Alternaria leaf spot, Collar rot and Phytophthora blight disease of pigeonpea crop.

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